Intra-population plasticity of *Anopheles darlingi*'s (Diptera, Culicidae) biting activity patterns in the state of Amapá, Brazil

Plasticidade intrapopulacional nos padrões de atividade hematofágica de *Anopheles darlingi* (Diptera: Culicidae) no Estado do Amapá, Brasil

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Keywords
Anopheles.# Ecology, vectors.# Insect vectors.# Malaria, vectors.# – *Anopheles darling*. Biting behavior. *Anopheles marajoara*.

Abstract

Objective
To assess the variation in *Anopheles darlingi*'s biting activity compared to *An. marajoara* in the same locality and to biting activity data from other regions.
Methods
Using human bait, eight observations of the biting activity of *An. darlingi* and *An. marajoara* were carried out during 1999 and 2000 in the municipality of São Raimundo do Pirativa, state of Amapá, Brazil. Each observation consisted of three consecutive 13-hour collections, close to full moon. There were shifts of collectors in the observation points and nocturnal periods.
Results
An. darlingi revealed considerable plasticity of biting activity in contrast to *An. marajoara*, which showed well-defined crepuscular biting peaks. No significant correlation between density and biting activity was found, but a significant correlation existed between time and proportional crepuscular activity, indicating underlying ecological processes not yet understood. Two of the four available data sets having multiple observations at one locality showed considerable plasticity of this species' biting patterns as well.
Conclusion
Intra-population variation of biting activity can be as significant as inter-population variation. Some implications in malaria vector control and specific studies are also discussed.

Descritores
Anopheles.# Ecologia de vetores.# Insetos vetores.# Malária, vetores.# – Anopheles darling. *Atividade hematofágica.* Anopheles marajoara.

Resumo

Objetivo
Examinar a variação no ciclo de atividade hematofágica de Anopheles darlingi *em uma localidade, em comparação com* An. marajoara *na mesma localidade e com dados de atividade hematofágica de outras regiões.*
Métodos
Durante 1999 e 2000 foram feitas oito observações da atividade de picar de An. darlingi *e* An. marajoara*, utilizando isca humana, na localidade de São Raimundo do Pirativa, Estado de Amapá, Brasil. Cada observação era composta de três coletas consecutivas de 13 horas, situadas ao redor da ocorrência de lua cheia. Os coletores foram trocados entre os pontos de observação e os períodos noturnos.*

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Resultados

An. darlingi *mostrou considerável plasticidade na atividade hematofágica, em contraste com* An. marajoara*, que sempre mostrou um pico crepuscular bem definido. Nenhuma correlação significativa entre densidade e atividade hematofágica foi encontrada, mas houve uma correlação significativa entre tempo e atividade proporcional crepuscular, indicando mecanismos ecológicos subjacentes ainda não compreendidos. Dois dos quatro conjuntos de dados, contendo observações múltiplas na mesma localidade, também mostraram considerável plasticidade na atividade hematofágica dessa espécie.*

Conclusão

Variações intrapopulacionais na atividade hematofágica podem ser tão grandes quanto as interpopulacionais. Algumas implicações para o controle de vetores de malária e de estudos específicos são discutidas.

INTRODUCTION

Knowing the biting activity patterns of malaria vectors is essential for accurately planning vector control actions. The biting behavior of *Anopheles darlingi* Root, 1926, main malaria vector in Amazon region, has been studied extensively throughout the last six decades. Great differences in biting activity patterns have been reported throughout the species' geographic extent, from very pronounced almost exclusively crepuscular to almost exclusively nocturnal activity.[4]

The variation of biting activity patterns contrasts to those reported for most other Anopheline mosquitoes in the region, that most often follows a crepuscular biting pattern.[8,13,14] This fact, in addition to other biological variation, has originated the hypothesis that *An. darlingi* is a species complex. Species complexes are common within the *Nyssorhynchus* subgenus of Anopheles. Of currently recognized or possible species complexes,[11] *An. albitarsis*, *An. triannulatus*, *An. oswaldoi*, and *An. nuneztovari*, only *An. nuneztovari* is known for its marked differences in biting activity patterns.[13] Genetic studies of *An. darlingi* have shown so far that this is a monotypic species.[11] And there is no evident geographical distinction of biting activity patterns.

The present study examines the plasticity of *An. darlingi*'s biting activity at one locality to assess whether the type of variation of biting behavior found at one site is present in the whole range. Another important Anopheles species, *An. marajoara*, collected at the same site and that shows a distinct crepuscular biting behavior, was used as a comparison to *An. darlingi*.

METHODS

Study area

The biting activity observations were carried out at the locality of São Raimundo do Pirativa, municipality of Santana, state of Amapá, Brazil (0°2' N, 51°15' W). Amapá has an equatorial hot and humid climate with temperatures varying between 22–32 °C. The rainy season extends from January until July with a mean monthly rainfall of 2,100 mm. The dry season extends from August until December with a mean monthly rainfall of 178 mm. The average relative humidity is 85%. The study site lies along the Matapí River in a flooded forest area ("várzea") with tidal influence. It is a small riverine community of approximately 200 inhabitants. Their main economic activities are fishing, small-scale extraction of forest products, small-scale agriculture, and per diem work in neighboring buffalo farms. Malaria transmission is intense and year-round and both *Plasmodium falciparum* and *P. vivax* are notified as vectors by the local health services. *Plasmodium malariae* occurs as well, evidenced through population immunological examination (36% IgG antibody prevalence) and CS-rate studies of the Anopheles species (Branquinho and Voorham*).

A recent study looking for the *An. albitarsis* complex in an area close to the study site revealed only the presence of *An. marajoara*. Therefore, analysis of the available data will be carried out assuming a one-species setting, and this species name will be used throughout the text. This species has recently been shown to be a potentially important vector, (Conn[5] and Branquinho and Voorham*) in the state of Amapá.

*On-going research, M.S. Branquinho and J. Voorham. Unpublished data.

Data collection and processing

Along seven consecutive months, from May to October 1999, and once in March 2000, three consecutive night collections were performed close to full moon. Local community adults were hired as mosquito collectors. These people have already been exposed to the circulating *Plasmodium* parasites, and were offered chemoprophylaxis according to the Ministry of Health guidelines. Mosquitoes landing on the bare legs of three human collectors outdoors were caught before biting and kept in separate collection cups for each observation hour. The observation night started 30 minutes before sunset and ended 30 minutes after sunrise. Since the study site is situated almost at 0° latitude, there were 13 hours of observation per night. There were two collection shifts: the first 6 hours of nighttime followed by the last 7 hours. Over the three nights of observation, there was a rotation of collectors regarding their shift periods and observation points. Immediately after the completion of an hour of observation, the species of collected mosquitoes were identified, and stored on silica gel for further infectivity studies, until 1 AM. Processing continued the next morning.

Data obtained from three consecutive nights of observation were lumped into a biting activity data set, thus correcting for eventual individual differences between collectors and collecting spots. Three-night observation is referred by a week number, the March 2000 observation is referred as a continuation of the 1999 week-count.

In May 1999 an age-composition study was done with the collected *An. darlingi* from the first 7 hours of the night, using parity observations following Detinova.

RESULTS

The results are summarized in Table 1 and show that *An. darlingi* (74.7%) and *An. marajoara* (22.7%) were the most abundant species collected. *Anopheles darlingi* showed a sharp increase in density by the end of the rainy season, while *An. marajoara* density was fairly constant throughout the study.

The observed biting activity patterns are shown in Figure 1 for *An. darlingi* and in Figure 2 for *An. marajoara*. A large variability in biting activity over the eight observations was observed for *An. darlingi*, contrasting to more stable patterns seen for *An. marajoara*. While the latter continuously showed a well-pronounced peak during the first three hours of the night, the former species varied considerably in its activity pattern. In addition, *An. darlingi* showed a more pronounced nocturnal activity than *An. marajoara*.

Two potential causes for the observed plasticity of *An. darlingi* were examined. First whether relative

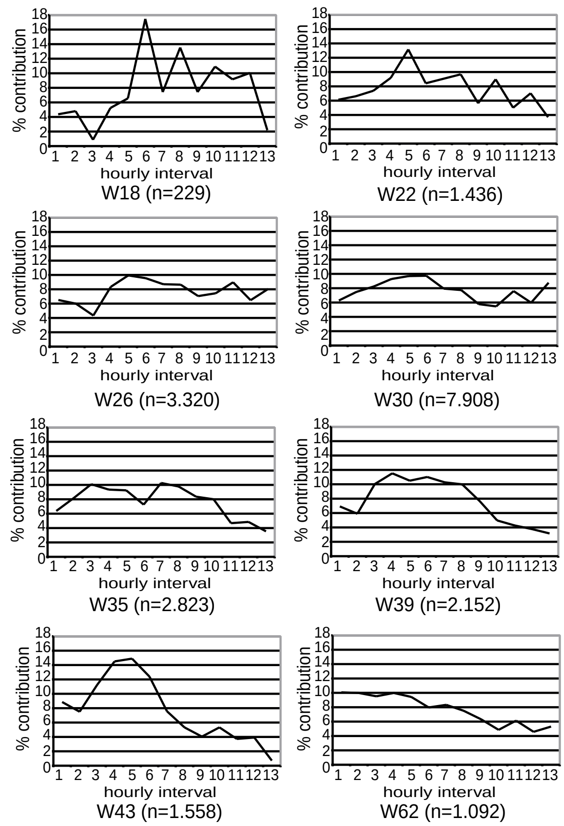

Figure 1 - Biting activity patterns of *An. darlingi* during the eight observations at São Raimundo do Pirativa, State of Amapá, Brazil, from May 1999 until March 2000.

Table 1 - Number of *Anopheles* specimen collected per three-day observation per species, at São Raimundo do Pirativa, State of Amapá, Brazil, from May 1999 until March 2000. Collections are coded according to the week number of the year 1999, with the March 2000 collection as a continuation of the 1999 numbering.

	Collection (week number)								Total	
Species	W18	W22	W26	W30	W35	W39	W43	W62	N	%
An. darlingi	229	1,436	3,320	7,908	2,823	2,152	1,558	1,092	20,518	74.7
An. marajoara	1,500	1,709	734	656	366	438	370	464	6,237	22.7
An. triannulatus	0	35	63	19	58	41	38	14	268	1.0
An. nuneztovari	0	43	55	80	41	16	15	146	399	1.5
An. braziliensis	0	2	0	4	6	8	14	0	34	0.1
An. oswaldoi	0	0	0	0	0	1	1	0	2	0.0
	1,729	3,225	4,172	8,667	3,294	2,656	1,996	1,716	27,458	100

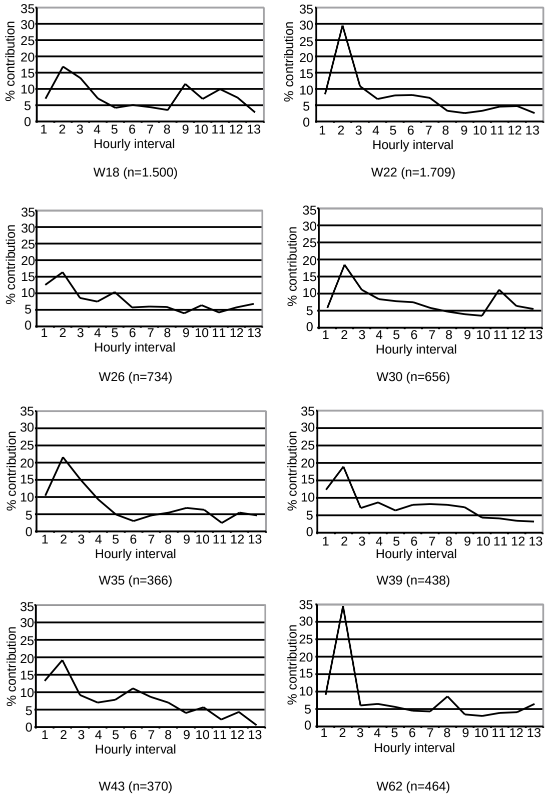

Figure 2 - Biting activity patterns of *An. marajoara* during the eight observations at São Raimundo do Pirativa, State of Amapá, Brazil, from May 1999 until March 2000.

density contributed to the variation. Results showed that there was no significant relationship between density and the proportional contribution of biting during the first three hours for *An. darlingi* (p=0.82) and *An. marajoara* (p=0.93).

Second whether there was a time of year effect on the collection. A significant positive correlation between week-number (*i.e.* time) and the proportional contribution of biting during the first three hours can be observed for *An. darlingi* (r=0.85, p=0.007) but not for *An. marajoara* (r=0.46, p=0.25).

The single parity observation was performed using 311 *An. darlingi* specimens divided over the first seven hours of the night, and showed no significant differences of parity-rate between the hours (χ^2, p=0.07), and a mean value of 66%.

DISCUSSION

Overall biting activity patterns of *An. darlingi* in the study is similar to the results reported for the state of Amazonas, Brazil,[6] French Guiana,[9] Colombia,* Suriname,[7] and Venezuela.**

Although these regions are located in the northern part of the continent, it does not imply a geographical determination of biting activity patterns. For instance, in the state of Rondônia, Brazil[8], occasionally this species has shown very little crepuscular activity, and in several localities in the northern state of Pará, Brazil[14,15]– relatively close to the study area –, this species has shown pronounced crepuscular biting activity.

Few studies have been conducted on the biting activity patterns of *An. marajoara*, partly due to difficulties in identifying the species-complex's members. In Venezuela[13] and Trinidad,[1] *An. albitarsis s.l.* (probably *An. marajoara*) showed similar activity patterns to the ones found in this study with a very pronounced crepuscular peak.

One of the most important aspects of a vector's biting activity is how it relates to human activity. Throughout rural South America, where malaria is mostly found, human activity is mostly outdoors during crepuscular hours and indoors during nocturnal hours. Therefore, to better compare human and Anopheline activities, the biting patterns in Figures 1 and 2 were converted to relative contributions of three functional periods: 1) the first three hours of the night; 2) the last three hours of dawn; and 3) the nocturnal period in between. This arrangement also facilitates comparing the same species in other regions and other species, given that different methodologies have been used in other studies regarding time-scales, study design, and collection methods. Figure 3 shows the results for both species (A and B). From these functional diagrams it can be drawn that *An. marajoara* has a much higher contribution to biting than *An. darlingi* when the human individuals are outdoors (Period 1).

Although in two out of eight observations *An. marajoara* showed lower densities than *An. darlingi*, its higher contribution to extra-domiciliar biting clearly indicates this species' importance as a potential malaria vector, especially where vector control measures are mainly house-based.

Of 34 studies examined, four also described local variation of the biting activity of *An. darlingi.* These studies were conducted in the state of Amazonas[6] and Rondônia,[8] Brazil, Honduras,[10] and Venezuela.** Figure 3 presents functional-period diagrams for each location. Local variation in biting activity by collection period was minimal in Venezuela (F) and Honduras (E), but considerable in Rondônia (D) and

*Suarez M et al. Unpublished data.
**Rubio-Palis Y. Unpublished data.

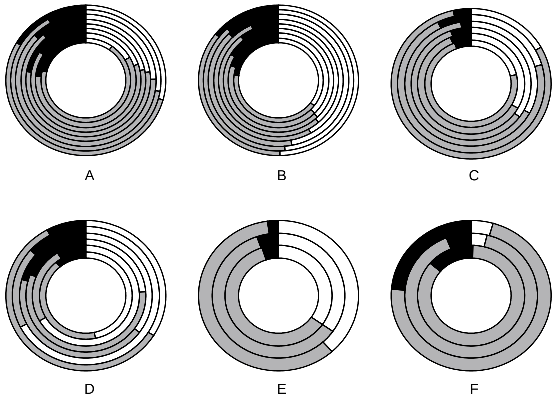

Figure 3 - Overview of variation in biting contribution in the three functional periods of the night. (A) Present study, *An. darlingi*; (B) Present study, *An. marajoara*; (C) Amazonas State, Brazil[6], *An. darlingi*; (D) Rondônia State, Brazil[8], *An. darlingi*; (E) Honduras[10], *An. darlingi*; (F) Santa Maria de Los Guiacas, Venezuela (Rubio-Palis, unpublished data), *An. darlingi*. White = first 3 hours, Gray = middle 7 hours, Black = last 3 hours.

Amazonas (C). Compared to Amazonas, the observed pattern in Amapá showed considerably more early morning biting activity, although early evening activity was similar. Early morning biting activity contribution in Rondônia was similar to Amapá, but the early evening crepuscular activity in Rondônia was much greater, but also more variable. Thus, behavioral plasticity is not always observed as seen in Venezuela and Honduras. However, other data obtained in the Amazon region clearly show that this is not a peculiarity of this study area.

The likelihood of mosquito density interfering with the biting behavior was discarded in the present study, as previously reported elsewhere in Brazil[2] and French Guiana.[9] However, studies conducted in Colombia* suggested a correlation between biting activity and the population density of *An. darlingi*. A regional study in Brazil also alluded to this possibility.[15]

Moonlight could have influenced the biting activity patterns and densities[12] of previously mentioned observations with local variation, but this was not the case for the present study since all collections were carried out during the same moon phase. A higher variability can be expected when not correcting for this influence.

The age composition of biting *An. darlingi* was shown to be different during the crepuscular peaks in Brazil,[2] opposing results have been found in other studies[4,9] as well as in the present one. Age-composition differences between activity periods do not necessarily indicate subgrouping, and it is more likely to be a result of specific ecological settings than of age-specific activity patterns. However, additional studies on age structure of *An. darlingi* and *An. marajoara* at the study site are intended, using more precise methods.

The observed correlation between time and the contribution to crepuscular biting for *An. darlingi* still requires further clarification, and indicates the existence of underlying ecological processes not yet understood.

Population variations throughout *An. darlingi*'s wide geographical range could explain the differences in biting activity patterns.[4] However, this does not provide an explanation for intra-population variationσ, which can be as extent as inter-population variations. It is beyond this study's scope to further discuss possible causes for *An. darlingi* being so plastic in its biting activity patterns on regional and local levels. Evidence up to date strongly indicates that ecological determinants play a role instead of genetics or local population specific characteristics. Further studies on population dynamics and individual mosquito's behavior are needed on the subject.

Plasticity in biting activity patterns can result in increased vectorial potential and control strategies may have to be adjusted to account for differences in human vector contact over time.

Knowledge of vector biting activity plasticity is also essential for specific entomological studies. Sampling assumes knowledge of the population's activity pattern. When biting pattern observations are limited to only a few nights of observation, the plasticity of this behavior can not be evidenced and could lead to misinterpretation of data. To illustrate that, a comparison between relative density estimates of whole-night and

Table 2 - Comparison of relative density and nocturnal period contribution between all-night observations and partial (hours 2-3) observations for *An. darlingi* and *An. marajoara* at São Raimundo do Pirativa, 1999-2000. Collections are coded according to the week number of the year 1999, with the March 2000 collection as a continuation of the 1999 numbering.

Collection	*An. darlingi*			*An. marajoara*		
		Duration			Duration	
	% 2-3*	13h	2h	% 2-3	13h	2h
W18	5.7	2.0	0.7	30.2	12.8	25.2
W22	14.0	12.3	11.2	40.3	14.6	38.3
W26	10.3	28.4	19.1	24.9	6.3	10.2
W30	15.7	67.6	69.1	29.6	5.6	10.8
W35	18.3	24.1	28.7	36.6	3.1	7.4
W39	15.9	18.4	19.1	26.0	3.7	6.3
W43	18.7	13.3	16.2	28.4	3.2	5.8
W62	19.5	9.3	11.8	40.5	4.0	10.4

*Percentage of biting contribution during the hours 2 and 3 of the night.

*Suarez *et al* – Unpublished date

2-hour periods (hours 2 and 3 – the most commonly used sampling period) is shown in Table 2, as well as the proportion of total biting during the 2-hour observation period for both studied species throughout eight observations. Around 6-20 % of all bites are due to *An. darlingi*, while 25-41% are due to *An. marajoara*. This difference in sampling could lead to bias due to period-specific characteristics.

The relative density estimates from partial collections are closer to all-night density estimates for *An. darlingi* than for *An. marajoara*, which are constantly overestimated. The combination of different sampling of the two species populations and overestimation of the relative density of one species could have lead to an overestimation of the importance of *An. marajoara* as a vector in a recent study in Amapá,[5] as it is likely to have occurred in other studies on multiple-vector settings.

In conclusion, inter-population variation in biting activity may be as great as intra-population variation. Variability in biting activity should be taken into account for intervention design and specific entomological studies, especially where there is vector heterogeneity. Comparison of one species populations in different areas, or two species populations at the same site should be done with sampling methods that minimize bias for density and age-composition estimates. It is also recommended to minimize the use of partial-night observations in situations where biting activity varies greatly.

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REFERENCES

1. Chadee DD. Indoor and outdoor host-seeking rhythms of *Anopheles albitarsis* (Diptera: Culicidae) in Trinidad, West Indies. *J Med Entomol* 1992;29:567-9.
2. Charlwood JD, Hayes J. Variações geográficas no ciclo de picada do *Anopheles darlingi* Root no Brasil. *Acta Amaz* 1978;8:601-3.
3. Charlwood JD, Wilkes TJ. Studies on the age-composition of samples of *Anopheles darlingi* Root (Diptera: Culicidae) in Brazil. *Bull Entomol Res* 1979;69:337-42.
4. Charlwood JD. Biological variation of *Anopheles darlingi* Root. *Mem Inst Oswaldo Cruz* 1996;91:391-8.
5. Conn JE, Wilkerson RC, Segura MNO, Souza RTL de, Schlichting CD, Wirtz RA, Póvoa MM. Emergence of a new neotropical malaria vector facilitated by human migration and changes in land use. *Am J Trop Med Hyg* 2001. In press.
6. Hayes J, Charlwood JD. Dinâmica estacional de uma população de *Anopheles darlingi*, numa área endêmica de malária no Amazonas. *Acta Amaz* 1979;9:79-86.
7. Hudson JE. *Anopheles darlingi* Root (Diptera: Culicidae) in the Surinam rain forest. *Bull Entomol Res* 1984;74:129-42.
8. Klein TA, Lima JBP. Seasonal distribution and biting patterns of *Anopheles* mosquitoes in Costa Marques, Rondonia, Brazil. *J Am Mosq Control Assoc* 1990;6:700-7.
9. Pajot F, Le Pont F, Molez J, Degallier N. Agressivité d'*Anopheles* (*Nyssorhynchus*) *darlingi* Root, 1926 (Diptera, Culicidae) en Guiane française. *Cah ORSTOM Ser Entomol Med Parasitol* 1977;15:15-22.
10. Rivera Núñez LA. *Algunos aspectos de comportamiento de* Anopheles darlingi *(Diptera: Culicidae) de la Ceiba, Atlántida [Tesis de maestría].* Honduras Panama: Universidad de Panamá; 1990.
11. Rosa-Freitas MG, Oliveira RL de, Carvalho-Pinto CJ de, Flores-Mendoza C, Fernandes Silva-do-Nascimento T. Anopheline species complexes in Brazil. Current knowledge of those related to malaria transmission. *Mem Inst Oswaldo Cruz* 1998;93:651-5.
12. Rubio-Palis Y. Influence of moonlight on light trap catches of the malaria vector Anopheles nuneztovari in Venezuela. *J Am Mosq Control Assoc* 1992;8:178-80.
13. Rubio-Palis Y, Curtis CF. Biting and resting behaviour of anophelines in western Venezuela and implications for control of malaria transmission. *Med Vet Entomol* 1992;6:325-34.
14. Tadei WP, Mascarenhas BM, Podestá MG. Biologia de anofelinos amazônicos. VIII. Conhecimento sobre a distribuição de espécies de *Anopheles* na região de Tucuruí-Marabá (Pará). *Acta Amaz* 1983;13:103-40.
15. Tadei WP, Thatcher BD, Santos JM, Scarpassa VM, Rodrigues IB, Rafael MS. Ecologic observations on anopheline vectors of malaria in the Brazilian Amazon. *Am J Trop Med Hyg* 1998;59:325-35.